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Activated Protein C Resistance and the Factor V Leiden Mutation in Children With Thrombosis

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To determine the prevalence of activated protein C resistance and the factor V Leiden mutation (position 1691, arginine 506 to glutamine substitution) in children with thrombosis, plasma samples from children with thrombosis were tested for activated protein C resistance. DNA was analyzed for the factor V Leiden mutation. Five of 34 children (15%) had activated protein C resistance; each was heterozygous for the factor V Leiden mutation. All 5 children heterozygous for the factor V Leiden mutation suffered non-CNS venous thromboses comprising 21% of the group of children (5/24) with non-CNS venous thrombotic events. Each of these 5 children had a family history of thrombosis. In conclusion, children with non-CNS venous thrombosis should be evaluated for the factor V Leiden mutation. Children most likely affected are those with a family history of thrombosis. Am. J. Hematol. 57:29–32, 1998.

Key words: activated protein C resistance; factor V Leiden mutation; thrombosis; children

INTRODUCTION

Recently, a single point mutation (position 1691, arginine 506 to glutamine) in the coagulation factor V gene has been identified as the most common genetic risk factor for thrombosis in adults [1–8]. This mutation resulting in production of a mutant factor V protein is termed factor V Leiden and has been found in 3–7% of the general population [2,8]. Twenty to 40% of adults with a first episode of venous thrombosis have the factor V Leiden mutation [1–3,9,10]. Up to 60% of adults with venous thrombosis and a family history of thrombosis are heterozygous for this mutation [3–5,7,9–11]. Screening for the factor V Leiden mutation can be performed by testing for the relative resistance of the abnormal factor V molecule to inactivation by activated protein C. APCR has been predictive of the factor V Leiden mutation in 78–100% of adults tested [4–7,12].

Earlier we showed that 70% of children with thrombosis had either a genetic deficiency of protein C or protein S and/or a lupus anticoagulant [13]. Here we expand what is known about risk factors for thrombosis in children by reporting on the prevalence of APCR and the factor V Leiden mutation in children with thromboses.

METHODS

Clinical Procedures

All children with thrombosis referred to the authors since April 1994 have been prospectively screened for APCR. In addition, stored frozen plasma samples were also available from 3 children with thrombosis seen before April 1994 and were also studied after informed consent was obtained. All thromboses were documented by imaging studies including: Doppler ultrasound, venogram, arteriogram, computerized tomography, and mag-

Abbreviations used: APCR, activated protein C resistance; APTT, activated partial thromboplastin time; bp, basepairs; CNS, central nervous system; INR, international normalized ratio; n-APCRr, normalized activated protein C resistance ratio.

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netic resonance imaging. Purpura fulminans was clinically diagnosed.

Family history was considered positive for thrombosis if any venous or arterial thrombotic events had occurred in the first- or second-degree relatives.

Laboratory Procedures

For APCR testing, blood was collected by a two-syringe technique into 3.2% buffered sodium citrate anticoagulant and transported on ice to the laboratory. The citrated plasma was centrifuged at 2,500*g* for 20 min at 4°C and the platelet-poor plasma frozen at −70°C until the time of assay.

The APTTs were performed on a ST4 coagulometer (American Bioproducts, Parsnippany, NJ) in duplicate. Plasma samples from 30 healthy, fasting adults who had not done strenuous exercise prior to testing, were not pregnant, and were not receiving any medications were used to establish the reference range for the standard APCR assay. This assay was performed as previously described [14]. Results are expressed as the normalized activated protein C resistance ratio (n-APCRr). This ratio is obtained by determining the activated protein C ratio of the test sample and dividing by the activated protein C ratio of the normal pooled plasma samples. This correction overcomes variables in test runs [15]. A ratio greater than or equal to 2 standard deviations below the lower limit of the normal range indicates resistance.

Plasma samples from study children were evaluated initially with either the standard assay or a modified version of the standard APCR assay since the standard assay for APCR cannot be used when the baseline APTT is prolonged [16]. The modified assay is based upon the one stage factor V assay as per Le et al. [16]. The 30 adult plasma samples were used as the control for the modified assay as well as the standard assay. Results are expressed as the modified n-APCRr.

Molecular analysis for the factor V Leiden mutation was performed on DNA isolated from whole blood collected in 3.2% buffered sodium citrate or EDTA and frozen unspun until the time of analysis. Genomic DNA was isolated from whole blood using commercially prepared reagents exactly as suggested by the manufacturer (Gentra Systems, Minneapolis, MN). To determine the presence of the factor V Leiden mutation, genomic DNA was amplified and assayed for loss of a cleavage site for the restriction enzyme *Mnl I* [4,11]. A portion of the factor V gene including the A to G substitution at nucleotide 1,691, which produces the Arg 506 to Gln amino acid substitution, was amplified by polymerase chain reaction using primers and reaction conditions as described by Ridker et al. [11]. The amplification product was digested overnight with *Mnl I* size fractionated by electrophoresis in 3% agarose gel and bands were visualized by ethidium bromide staining. Using this technique, a person with two wild type factor V alleles displays bands of 37, 82, and 104 bp, whereas those who are heterozygous for the factor V Leiden mutation exhibit bands of 37, 82, 104, and 141 bp. Persons homozygous display bands of 82 and 141 bp.

Molecular analysis for the factor V Leiden mutation was performed on each of the 30 control plasma samples. Study samples with either a standard or modified n-APCRr more than two standard deviations below the control mean had DNA analysis for the factor V Leiden mutation.

RESULTS

One of the 30 controls had an APTT of 27.4 sec with a mean n-APCRr of 0.66 and a modified n-APCRr of 0.26. DNA analysis revealed that this person was heterozygous for the factor V Leiden mutation. The remaining 29 controls had a mean APTT of 29.5 sec (SD 3.0 sec). Their mean n-APCRr was 0.98 (SD 0.12). Their mean modified APCRr was 1.15 (SD 0.25). None had the factor V Leiden mutation.

The study group was comprised of 34 children (19 boys) with a mean age of 8.9 years (range: newborn to 18 years). Their ethnicities were: 27 Caucasian, 3 Hispanic, 2 African-American, and 2 Native American. Twenty-four children had non-CNS venous thromboses including: 17 proximal veins, 2 distal veins, 2 purpura fulminans, 1 renal vein, 1 superior vena cava, and 1 primary pulmonary embolus. Nine of the 24 children had a venous access device associated thrombosis. Ten children had CNS thromboses; 6 were arterial, 1 venous, and in 3 children, without further studies, the vascular site of origin was indeterminant.

Twenty-five children had an APTT that fell within the control established normal range and were evaluated for APCR with the standard assay. Of the 25 children with a normal APTT, the four described in Table I had a n-APCRr that was more than 2 standard deviations below the control mean. Their n-APCRs were 0.58, 0.52, 0.56, and 0.67, respectively. They were reevaluated by the modified assay and their modified n-APCR's were below 0.64 (more than 2 standard deviations below the control mean). These results are shown in Table I. Each of these children proved to be heterozygous for the factor V Leiden mutation.

Nine children had a prolonged APTT, 4 receiving warfarin therapy, 4 with a lupus anticoagulant, and 1 neonate. Eight of these 9 children had non-CNS venous thromboses and 1 had a CNS thrombosis. Eight children demonstrated responses on the modified assay that fell within the normal range and on molecular analysis none had the factor V Leiden mutation. Child 5 in Table I had a modified n-APCRr of 0.56 and on DNA analysis was found heterozygous for the factor V Leiden mutation.

TABLE I. Children with the Factor V Leiden Mutation*

Child	Age (year) at thrombosis	Predisposing conditions	Family history for thrombosis	Site of thrombosis (venous)	Modified n-APCRr
1	8	Acute lymphocytic leukemia, central line	Positive	Superior vena cava	0.58
2	10	Protein S deficiency hyperlipidemia	Positive	Iliofemoral	0.52
3	16	Trauma	Positive	Popliteal	0.28
4	17	Anatomic defect, hyperlipidemia	Positive	Axillary-subclavian	0.54
5	14	Lupus anticoagulant	Positive	Femoral	0.56

*Modified n-APCRr: modified normalized activated protein C resistance ratio.

Nine of the 34 children (26%) had a family history of thrombosis; all had non-CNS venous thromboses. Five of the 9 children (56%) with non-CNS venous thrombosis and a positive family history of thrombosis had the factor V Leiden mutation. None of the children with CNS thromboses had a family history of thrombosis or the factor V Leiden mutation.

The frequency of the factor V Leiden mutation in our general population is 6% as determined by analysis of 115 random cord blood samples. There was a 100% correlation between the factor V Leiden mutation and a modified n-APCR result below two standard deviations of the mean control value in that series [17]. The odds ratio for the factor V Leiden mutation in children with non-CNS venous thrombosis is 2.72 (95% confidence interval 1.34–5.53). The *P* value is <0.005.

Information about the five children heterozygous for the factor V Leiden mutation is shown in Table I. Children 1, 2, 4, and 5 are Caucasian and child 3 is Native American. The children with the factor V Leiden mutation did not differ from those without the mutation in age, general health, or sites of thrombosis. Each had a non-CNS venous thrombosis; only one was associated with a venous access device. Each child had normal protein C and antithrombin III activities. As indicated, 1 of the 5 children was heterozygous for protein S deficiency. Each of the children had a positive family history of thrombosis. Family members of 4 of the 5 children were studied and found with the factor V Leiden mutation. Three of the 5 children and their family members have been previously reported [14].

Treatment for each child's thrombosis was at the discretion of the primary hematologist caring for the child. Each of the 5 children with the factor V Leiden mutation was treated with systemic urokinase (4,400 U/kg intravenous bolus, then 4,400 U/kg/hr for 48 h) and heparin (10 U/kg/hr for 48 h, then therapeutic heparin for 5–8 days). All then received warfarin to achieve an INR of 2.0–3.0. Child 1 continued to receive warfarin until he completed chemotherapy and his central venous catheter was removed; he has not experienced a recurrent thrombotic event in the subsequent 12 months. Child 2 remains anticoagulated given his concomitant protein S deficiency. Child 3 was not studied for the factor V Leiden mutation at presentation with the popliteal thrombus. Following completion of 6 months anticoagulation for that event, his warfarin was discontinued and he subsequently developed a femoral vein thrombosis and pulmonary embolus. He was then diagnosed with the factor V Leiden mutation. Child 4 underwent surgical correction of a thoracic outlet obstruction and discontinued warfarin 3 months postoperatively. She has been thrombosis-free in the ensuing year. Child 5 had recurrent femoral thromboses before recognition of the factor V Leiden mutation. Child 3, because he had recurrent thromboses, and child 5, due to the lupus anticoagulant in conjunction with the Factor V Leiden mutation, have been maintained on warfarin anticoagulation and done well.

DISCUSSION

The factor V Leiden mutation is the most common heritable condition predisposing to non-CNS venous thrombosis in adults [1–8]. In our series, 21% of the children with non-CNS venous thrombosis series were found heterozygous for the factor V Leiden mutation. The only published study of the frequency by APCR/the factor V Leiden mutation in children with thrombosis reports a 47% prevalence rate in their series of 15 children with non-CNS venous thrombosis [18]. Both 21 and 47% are consistent with the reported prevalence rates of the factor V Leiden mutation in adults with thrombosis [19].

Thrombosis in individuals with the factor V Leiden mutation often occurs in association with other genetic prothrombotic conditions or acquired triggering factors. Each of the children in our series with the factor V Leiden mutation had coexisting medical risk factors for thrombosis. In the study by Nowak Gottl et al., 43% of the children with the factor V Leiden mutation had exogenous risk factors for thrombosis [18]. The difference in the frequency of coexisting risk factors for thrombosis

in the two series may be due to the relatively small study populations in each and/or variability in the referral populations.

A positive family history of thrombosis is often identified in individuals with the factor V Leiden mutation. Approximately one-half the children in our series with a family history of thrombosis had this mutation. This prevalence rate is nearly identical to that reported for adults (50%) with venous thrombosis and a family history of thrombosis [8].

Whether the factor V Leiden mutation is associated with CNS thromboses in adults is unclear. Nowak Gottl et al. found the factor V Leiden mutation in 8 of 20 children with CNS thrombotic events [18]. In contrast, of the 10 children with CNS events we studied, none were affected. More children with CNS thromboses will need to be evaluated before it can be determined whether or not the factor V Leiden mutation is associated with CNS thromboses.

In summary, our data suggest that all children with a non-CNS venous thrombosis, particularly if there is a positive family history for thrombosis, should be tested for the factor V Leiden mutation. This should be in addition to testing for a lupus anticoagulant and deficiencies of protein C and protein S as we previously recommended [13]; coexistence of a protein C or protein S deficiency has been shown to significantly increase the risk of thrombosis in association with the factor V Leiden mutation [19–21]. If a child were found with the factor V Leiden mutation, testing for ATIII deficiency would be warranted since, similar to heterozygosity for protein C or protein S deficiency, coexistence of ATIII deficiency has been shown to increase the risk for thrombosis in persons with the factor V Leiden mutation [22]. Whether or not to first screen for APCR and proceed to DNA analysis if the ratio is low or to directly perform DNA analysis for the factor V Leiden mutation remains controversial [23].

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